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A comparative histomorphological and histochemical study of the goblet cells and Brunner's glands in the duodenum of rabbits and rats

Israa Jawad[1]; Khalid Hadi Kadhim , Diyar M. H. Kadhim[2]; Dina Hamid Sadiq[3]
[1]Department of physiology, Biochemical and Pharmacology / College of Veterinary Medicine/ AL-Muthanna university
[2] Department of Anat. and Hist./ College of Veterinary Medicine/ AL-Muthanna university
[3]Dep. of Anatomy/ College of Nursing/ university Al-Basra

Abstract

The purpose of this study was to describe, comparison the histological structures, histochemical features and distribution of the goblet cells and Brunner's glands in the duodenum of the rabbits and rats .The study was carried out on Ten samples of duodenum of each of rabbits and rats , immediately after slaughtering. The specimens were divided into cranial ,descending and ascending parts .five specimens was taken from each part of duodenum of each of the rabbits and rats and fixed in 10% formalin for 24 hours and treating by routine histological technique. Sections were stained by Hematoxylin & Eosin ,Picric acid stain and Alcian blue PH 2.5 stains. Mean length of crypts of Lieberkuhn and number of each crypts, goblet cells and alveoli of Brunner's glands in duodenum of rabbits were more than that in the duodenum of rats . The mean length and number of crypts of Lieberkuhn was increase toward the last parts of the duodenum in each of the rabbits and rats. Goblet cells were globular cells between the columnar cells in the epithelium lined each of the villi and crypts of Lieberkuhn in the tunica mucosa of the duodenum in rabbits and rats and take positive reaction each of PAS and Alcian blue pH 2.5 stains. The mean number of goblet cells in crypts of Lieberkuhn in duodenum each rabbits and rats were increased toward the jejunum. Brunner's glands were branched tubuloalveolar glands in the submucosa of each duodenal parts in rabbits , while absent in ascending part of rats duodenum, and these glands in rabbits duodenum were composed of two types of the cells (serous and mucous),while in rats duodenum only mucous cells, and stained with PAS stain only in duodenum of each of the rabbits and rats. The mean number of the alveoli of the Brunner's glands in the cranial part were more than that in other parts and decreased toward ascending part in rabbits duodenum ,while absent in ascending part of rats duodenum..

Introduction

The rabbit and rat are a good experimental clinico-anatomical model in the research of number of morphological diseases in humans and animals (1-5). Rabbits are used in wide range of commercial purposes such as meat in France, Spain, Italy and some middle East countries; as hair in production of coats , regal dresses and in medical research, they are referred to as hind-gut fermenters because of the cecum (6).The intestinal tract of mammals have two basic functions, facilitates easy absorption of nutritive materials, and act as barrier against microorganisms and different antigens includes lymphocytes, macrophage, plasma cells and paneth cells and their found to be largest endocrine gland in the body in term of both hormones and number of endocrine cells (7-9). The small intestine of rabbit is a highly complex structure ,lies in the right side of abdomen, approximately 12% of the gastrointestinal volume and divided into duodenum, jejunum and ileum .,that processes and digests food, largely with the help of a huge population of bacteria. The right lobe of pancreas is situated in the mesoduodenum of the duodenal loop. The luminal surface of the small intestine is covered by villi, enterocytes that cover over 90% of the cell population on the villi surface, The luminal surface of the enterocytes contains microvilli (4).The duodenum is relatively long in the rabbit. At its origin, the duodenum forms an acute angle with the pylorus, lies close to the liver, and is subject to compression by the liver.its started at the pylorus with a cranial portion, that forming a duodenal ampulla, descending part of duodenum is fixed to the pars intermedia of the ascending colo, the ascending part is fixed to the descending colon. The bile duct opens near origin of the duodenum ,pancreatic duct opens into the duodenum in about its terminus (5). The duodenum is responsible for further processing of the material from the stomach by secreting enzymes which are vital for digestion; it also mixes the digesta with these enzymes within its lumen. The primary functions of the small intestine are digestion and absorption ,by mixing of the food with digestive enzymes secreted from the endocrine glands, Most of the digestion of carbohydrates and simple proteins takes place in the duodenum (10).

They are simple tubular glands in most mammals , extending from the muscularis mucosa through the thickness of the lamina propria and they open into the intestinal lumen at the villi base , in the lining epithelium of these glands are found stem cells, absorptive cells, goblet cells, paneth cells , and some enteroendocrine cells (9-11).Most studies on histology of the duodenum regard Brunner glands, these glands were discovered by John Jacob Wepter and named after the swiss anatomist Conard Brunner who first described these glands in 1687, Brunner's glands are

specific to mammals (13) ,they are as mucous glands and some time serous glands ,their lies in submucosa of the duodenum (12-16). Brunner glands are produce an alkaline secretion that is capable to neutralizing the chymo acid of the stomach, and supporting favorable pH conditions for adequate action by pancreatic juice enzymes (17-19).

Materials and method

The study was performed using 10 of each healthy adult local breeds rabbit *(Oryctolagus cuniculus*) and rat(*norvegius rat*) during January and February 2018. They were euthanized with an overdose of ketamine administered intramuscularly. The ventral abdominal wall of each animal was removed, the intestinal tract was separated after sectioning the pylorus just before the duodenum, Each duodenal specimen was divided into three parts (cranial, descending and ascending) immediately after slaughtering was washed with normal saline solution (0.9%), ten specimens ($1cm^3$) from different regions of each part of the duodenum were taken and fixed by 10% formalin approximately 24 hours at room temperature, and treated by routine histological processing (20). The stains were used , Harries Hematoxylin and Eosin (H&E) for the general histological components , Periodic Acid Schiff (PAS) for carbohydrates and Alcian blue pH 2.5 for carboxylated and sulphated glycoconjugate acids. The length of crypts of Lieberkuhn , counts each of crypts of Lieberkuhn, goblet cells in the crypts of Lieberkuhn and alveoli of the Brunner's glands in tunica submucosa (X 40) in ten sections in each section used Five microscopic fields of each part of the duodenum. The mean (X-) and the standard error (S.E) were calculated for ten slides for each part of the duodenum (21).

Results

The duodenal wall of each rabbits and rats was composed of the four layers or tunicae (mucosa, submucosa, muscularis and serosa or adventitia), the duodenum of rabbits and rats not have plicae circulares and a poorly developed muscularis mucosae (Fig.1-4), mean length of crypts of Lieberkuhn in each part of the duodenum in rabbit was longer than that in rat ,mean length of crypts in cranial , descending and ascending parts of rabbit duodenum were (213.5 ±13.2) µm (221.4 ±10.1) µm (224.6 ±17.3) µm respectively, while in cranial ,descending and ascending parts of rat duodenum were (192.1 ±10.3) µm (200.6 ±9.4) µm (204.3 ±14.8) µm respectively (Table 1) , mean number of crypts in cranial , descending and ascending parts of rabbit duodenum were (6 ±1) (9 ±2) (13 ±1) respectively, while in cranial ,descending and ascending parts of rat duodenum were (4 ±1) (8 ±3) (11 ±2) respectively (Table 1) . Goblet cells

were unicellular mucous globular shaped between of the columnar cells in the epithelium lined each of the villi and crypts of Lieberkuhn in tunica mucosa of the duodenum each rabbit and rat (Fig. 4-6) , the mean number of goblet cells was increase toward the ascending part of duodenum in each rabbit and rat ,and mean their number in cranial, descending and ascending parts of rabbit duodenum were (42±5) (57±3) (87 ±11) respectively, while in cranial ,descending and ascending parts of the rat duodenum were (37 ±7) (49 ±5) (82±5) respectively (Table 1).The goblet cells were take positive reaction with PAS and Alcian blue pH 2.5,while negative reaction with H&E in duodenum each rabbit and rat (Fig.4-6).

Brunner's gland showed as branched coiled tubules lined by serous and mucous cells in rabbit duodenum and were composed of each the serous and mucous acini densely packed in the submucosa of duodenum, while only mucous cells in rat duodenum were only mucous acini (Fig.2,7,8) and separated into lobules by interlobular connective tissues (Fig.2,7), their found in the submucosa of each duodenal parts in rabbit ,while absent in the ascending part of rat duodenum (Table 1), the Brunner's glands stained with PAS only in duodenum each of rabbit and rat (Fig.8).The ducts of these glands penetrate the muscularis mucosa , ascend through the lamina propria and empty into the base of intestinal glands (Fig.9), the mean number of alveoli of the Brunner glands in cranial, descending and ascending parts of the rabbit duodenum were (38±7),(21±1) and (12±2) respectively, while in cranial and descending parts of the rat duodenum were (26±5) (17±3) respectively (Table1).

Table (1): The mean length of crypts µm ,number of each crypts ,goblet cells and alveoli of the Brunner's glands (X40) per microscopic field in duodenum of rabbit and rat (Mean ± S.E)

Measure / Part	Crypts		Goblet cells	Alveoli
	Length µm	number		
Cranial part in rabbit	213.5 ± 13.2	6 ± 1	42 ± 5	38 ± 7
In rat	192.1 ± 10.3	4 ± 1	37 ± 7	26 ± 5
Descending part in rabbit	221.4 ± 10.1	9 ± 2	57 ± 3	21 ± 1
In rat	200.6 ± 9.4	8 ± 3	49 ± 5	17 ± 3
Ascending part in rabbit	224.6 ± 17.3	13 ± 1	87 ± 11	12 ± 2
In rat	204.3 ± 14.8	11 ± 2	82 ± 5	-

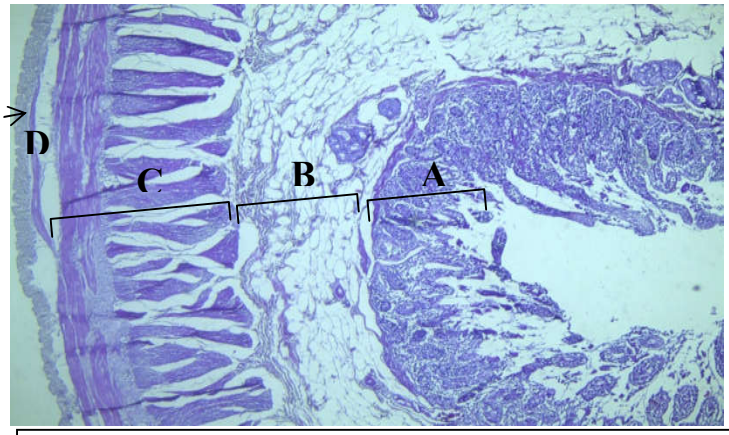

Fig.(1): histological section of duodenum of rat Showing: A-Mucosa, B.Submucosa, C .Muscularius, D.Serosa, H&E 40X

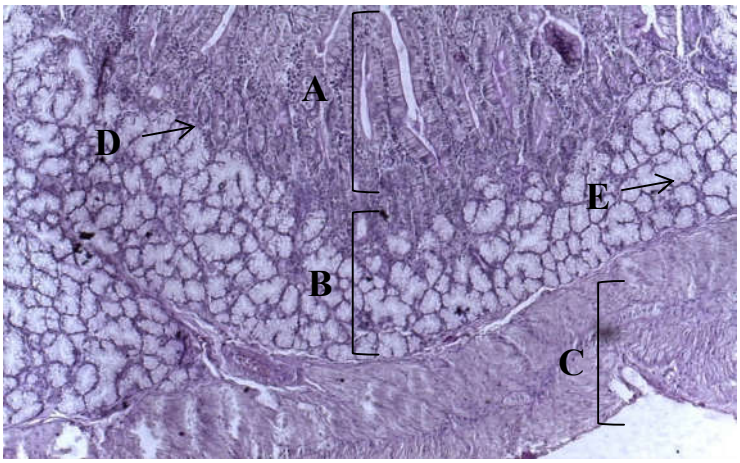

Fig.(2): histological section of duodenum of rabbit Showing: A-Mucosa, B.Submucosa, c .Muscularius, D.Muscularis mucosa, E. Brunner glands, H&E 100X

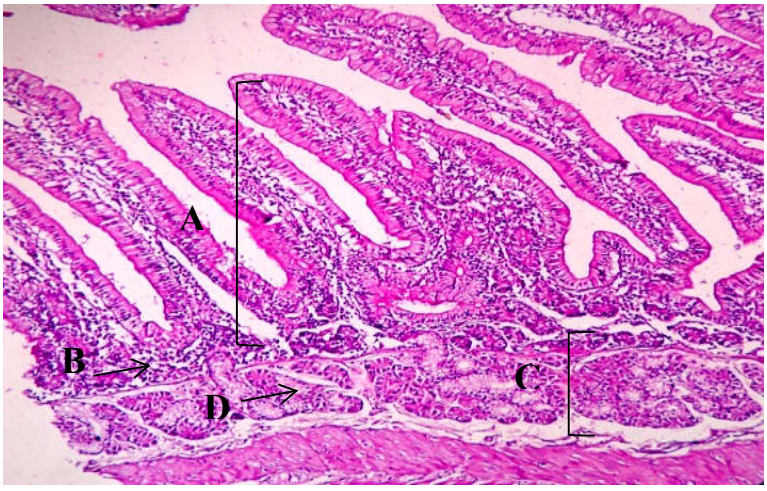

Fig.(3): histological section of duodenum of rabbit Showing: A-Mucosa, B..Muscularius mucosa,C. Submucosa, D.Brunner glands, H&E 100X

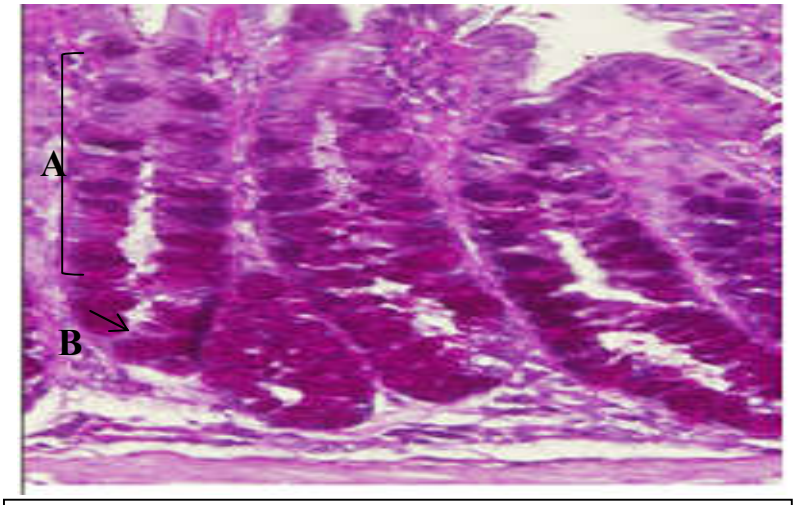

Fig.(4): histological section of duodenum of rat Showing: A-Crypts, B.goblet cells, PAS 400X

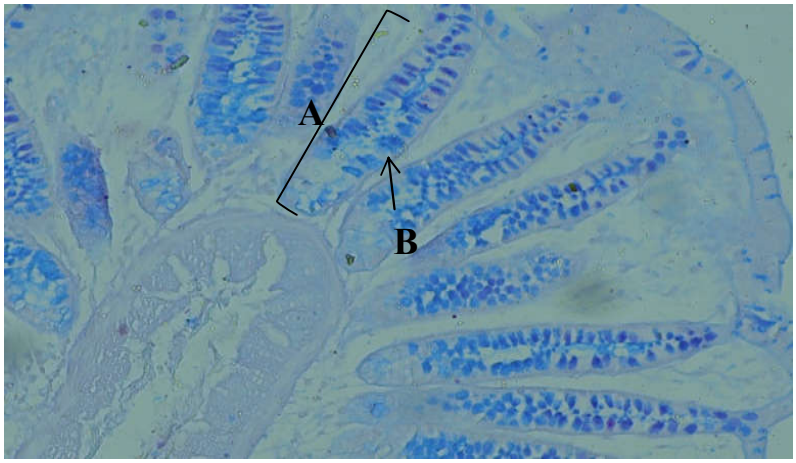

Fig.(5): histological section of duodenum of rabbit Showing: A-Crtpts, B.goblet cells, Alcian blue,PH 2.5 100X

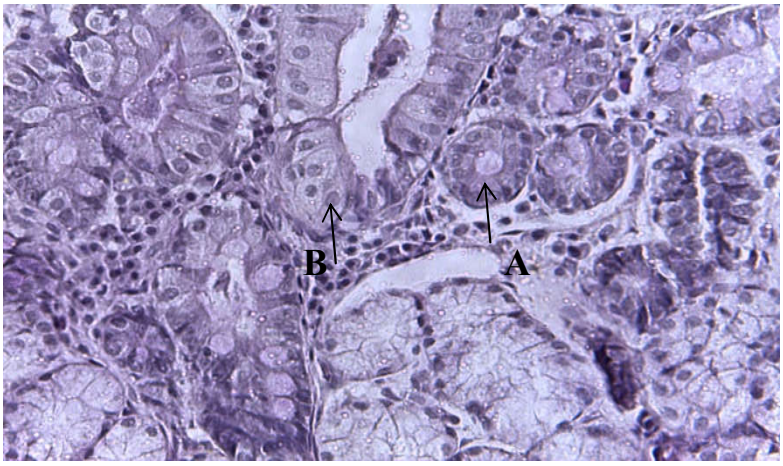

Fig.(6): histological section of duodenum of rabbit Showing: A-Crypts, B. goblet cells, H&E 400X

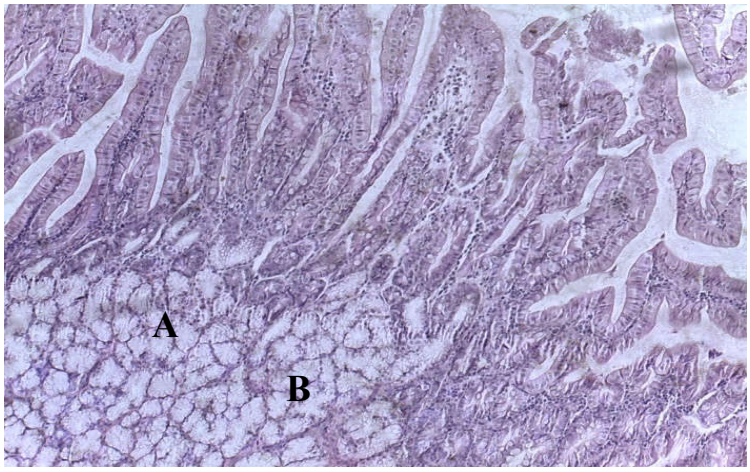

Fig.(7): histological section of duodenum of rabbit ,Showing: A- Brunner glands, B. submucosa, H&E 400X

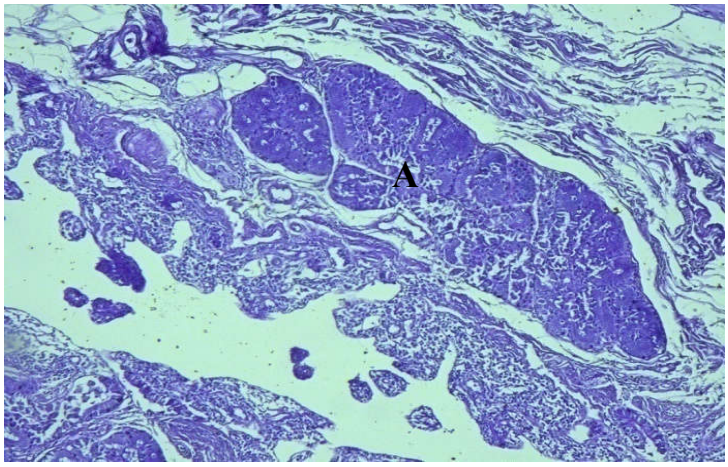

Fig.(8): histological section of duodenum of rabbit Showing: A-Brunner glands PAS 400X

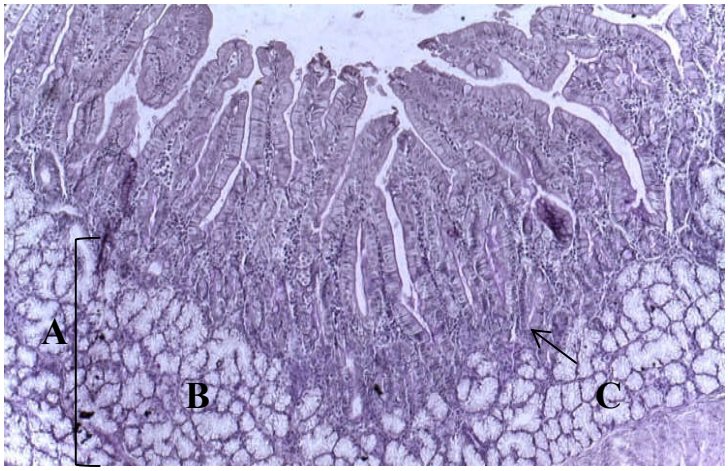

Fig.(9): histological section of duodenum of rabbit ,Showing: A- Brunner glands, B. submucosa,C.duct of Brunner glands H&E 400X

Discussion

The epithelial cells that line the duodenum are protected from harsh environment of acid, proteolytic enzymes, and abrasives in the lumen by a mucus layer ,these mucins are secreted by goblet cells and Brunner's glands (7). Mean number and length of crypts were increase toward the jejunum, the bases of the crypts showed considerable number of goblet cell , Goblet cells reside throughout the length of the small and large intestines and these cells increased progressively from duodenum to ileum ,so the amount of mucus varies from one species to another(9). Many studies explained the dietary factors effect goblet cell numbers and modulate the secretory activity of goblet cells(7), in each rabbit and rat the mean number of the goblet cells was increase toward the last parts of the duodenum , this finding was in accordance with (2,3). The major function of goblet cells is to lubricate and protect mucosal epithelia from damage caused by food, digestive secretions, the secretion of goblet cells is alkaline mucus for neutralize of the ingesta in cooperation with Brunner's glands to protect the duodenal lumen and assist the process of digestion and absorption by maintained an appropriate liquid state of the ingesta and microorganisms, also serves as a selective barrier for absorption across the intestine (7).

In the cranial part of the duodenum in each rabbit and rat the Brunner glands are massively present than that in the other parts, and there were decreased in the quantities toward the jejunum, and absent in ascending part of rat duodenum ,this is same to (22,23), The distributed of the Brunner glands several mammals starting from the gastrointestinal junction and extending to varying distances in the proximal small intestine (24-32), Herbivores have the longest distribution, omnivores a moderate distribution, and carnivores a short distribution(7). In rats the area extends one half ways down to the entry of the bile duct (23). In rabbits to extend near the jejunum (17,25).In guinea pig only of mucous acini and the glands were well developed in the cranial part of the duodenum (30), In primates extend distally along the intestinal tract(28) ,in *Didelphis virginia* confined to a very narrow region, immediately distal to the pyloric sphincter (33), suggest the cranial part of duodenum need for greater amounts of alkaline secretion from these glands in this part for aim of neutralize of the acidic chyme come from the stomach(32).The Brunner's glands assist the function of the crypts of Lieberkuhn in transporting immunoglobulin into the duodenal lumen. In addition, the presence of lysozyme in the cells of the secretory units of Brunner's glands continuously secrete bactericidal enzyme in human (33,34),there are numerous groups of serous-type cells lying among the mucous acini in rabbit. It has long been believed that these cells are of a nature similar to those of the

pancreatic acini, while they were demonstrated to be composed of only mucous cells in rat, this similar to (24) ,the secretory units of Brunner's glands in the raccoon and mouse were more dilated and lined with flatter cells (34).In bison, deer, guinea pigs, vole and domestic rabbits, they contain acidic sulphated and carboxylated mucins, whereas in humans, rhesus and Japanese macaques, cats, raccoons, rats, and opossums, they contain neutral mucins. This variation not be attributed to either the order or the diet of the mammals, although rats and voles are both rodents, Brunner's glands in the rat produce only neutral mucins, while in the vole produce acidic mucins. The existence of duodenal sub mucosal glands in the duodenum is uncontestable (7,24). The Brunner's glands of guinea pig were composed of only mucous acini,the secretion produced is a mixture of both acid and neutral mucins (18) The ducts of these glands penetrate the muscularis mucosae and usually pierce the base of the crypts of lieberkuhn to deliver their secretory product into the lumen of the duodenum (30),main secretory product of these glands is mucin which protects the duodenal mucosa by neutralizing acidic chyme from stomach, Mucins have been referred to as mucopolysaccharide and glycosaminoglycans (29)and possibly by the buffering capacity of its bicarbonate content ,also provides optimal conditions for enzyme action on pancreatic juices (32). These secretions are respond to parasympathetic vagal stimuli (14).

Brunner's glands of the rabbit and rat when treated with alcian blue pH 2.5 stained take a negative reaction, indicating the presence of substantial amount of acid mucins ,but positive with PAS staining revealed the presence of amount of mucin in the secretion of Brunner's glands . similar to this was obtained by (24). staining properties of Brunner glands are marked differences ,in bison, deer, voles and domestic rabbit they contain acidic sulphated and carboxylate mucins, whereas in humans, cats, raccoons and rats they contain neutral mucins. this variation could not be attributed to either order or the diet of the mammals (31). (30)found that in guinea pig, the deeper glands contain abundant sulfomucins with some neutral mucins. These glands contain equal amounts of neutral and sialomucins ,intense staining of guinea pig Brunner's glands with PAS indicating the presence of substantial amount of neutral mucin,When AB pH 2.5 – PAS technique was employed Brunner's glands stained variably with blue and purple indicating presence of mixture of acid and neutral mucins. More intensity of blue indicates higher amount of acid mucin and moderate neutral mucin (14).**Conclusion**: The present study showed reversed relation between number of the crypts, goblet cells and the Brunner's glands for neutralize of the ingesta and available the mucosal immunity to protect of the duodenal lumen against the erosive effects of the gastric juice by virtue of the mucoid nature of its secretion.

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دراسة شكلية نسجية وكيمياء نسجية مقارنة للخلايا الكاسية وغدد برونر في عفج الأرانب والجرذان

خالد هادي كاظم ديار محمد حسين [١]

[١]قسم التشريح والأنسجة /كلية الطب البيطري /جامعة المثنى

Email:dr.kh2017@gmail.com , Phone number: +9647819722624

الخلاصة

تهدف الدراسة الحالية لوصف ومقارنة التراكيب النسجية والخصائص الكيميائية النسجية وتوزيع الخلايا الكاسية وغدد برونر في العفج للأرانب والجرذان . أجريت الدراسة على عشر عينات من العفج لكل من الأرانب والجرذان بعد الذبح مباشرتا . قسمت العينات إلى جزء إمامي وجزء نازل وجزء صاعد،تم قطع خمس عينات من كل جزء من عفج كل من الأرانب والجرذان وثبتت في محلول الفورمالين(١٠%) تقريبا ٢٤ ساعة بعد ذلك مررت العينات بالتقنية النسجية الروتينية ،لونت الشرائح النسجية بملون الهارس هسماتوكسيلين والايوسين وكاشف شف الدوري وملون السيان الأزرق(اس هيدروجيني ٢.٥) متوسط طول خبايا لايبركن وعدد كل من الخبايا والخلايا الكاسية واسناخ غدد برونر في عفج الأرانب أكثر مما هي عليه في عفج الجرذان، متوسط طول وعدد خبايا لايبركن ازداد باتجاه الأجزاء الاخيره للعفج في كل من الأرانب والجرذان.الخلايا الكاسية ظهرت بشكل كروي بين الخلايا العمودية في الغشاء الظهاري المبطن كل من الزغابات وخبايا لايبركن في الغلالة المخاطية لعفج كل من الأرانب والجرذان وأخذت تفاعل موجب لكل من

كاشف شف الدوري وملون اليسان الازرق. متوسط عدد الخلايا الكاسية في حبايا لايبركن في عفج كل من الأرانب والجرذان ازداد باتجاه الصائم.غدد برونر تكون غدد سنخيه انبوبيه متفرعة في الغلالة تحت المخاطية لكل اجزاء العفج في الارانب لكن اختفت في الجزء الصاعد لعفج الجرذان ، وهذه الغدد في عفج الأرانب تتكون من نوعين من الخلايا (مصلية ومخاطية)،لكن في عفج الجرذان فقط خلايا مخاطية وتلون فقط بكاشف شف الدوري في كل من الارانب والجرذان. متوسط عدد اسناخ غدد برونر في الجزء الامامي تكون اكثر مما هي في الأجزاء الاخرى وتقل باتجاه الجزء النازل لعفج الأرانب ولكن تختفي في عفج الجرذان. نستنتج انه الدراسة الحالية أظهرت وجود اختلاف في إفراز غدد برونر بين الارانب والجرذان ووجود علاقة عكسية بين عدد كل من خبايا لايبركن والخلايا الكاسية وغدد برونر في عفج كل من الأرانب والجرذان لمعادلة المواد المهضومة وتوفير وظيفة مناعية .

الكلمات المفتاحية: خبايا لايبركن،الخلايا الكاسيه، غدد برونر، مقارنة ، الارانب والجرذان